Platelet Transfusion Therapy for Alloimmunized Patients: Selective Mismatching for HLA B12, an Antigen With Variable Expression on Platelets

By Charles A. Schiffer, Bernadette O’Connell, and Edward J. Lee

There can be wide variation in the expression of the HLA B12 antigen and its “splits” B44 and 45, on the platelets and lymphocytes from the same individual. One hundred sixty-two single donor platelet transfusions mismatched only for this antigen group were administered to 54 alloimmunized patients who were refractory to random donor platelets. Satisfactory increments (one-hour post-transfusion corrected-count increment [CCI] > 7,500) were seen following 111/162 transfusions (89%). In 31 patients (57%), all transfusions (n = 85) produced CCI > 7,500, and 76% of patients received some transfusions that were satisfactory. Of note is that ten patients had excellent increments despite either preformed lymphocytotoxic antibody against the mismatched antigen or positive lymphocytotoxic cross-matches with the donor. In contrast, poor increments were seen in ten recipients under similar circumstances, implying disparities in antigen expression on the platelets and lymphocytes of different donors. There was no obvious pattern of other donor HLA antigens which could be correlated with these differences. The HLA B12 antigen group is relatively common (found in approximately 25% of the population), and these data indicate that selective mismatching for these antigens can be an effective donor-selection strategy to increase the number of donors for alloimmunized recipients.

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Although donor selection by HLA typing of lymphocytes represents the most satisfactory means of providing platelet transfusions for alloimmunized recipients, approximately 30% to 40% of such transfusions fail to produce satisfactory post-transfusion increments. Results are generally better if the donor and recipient are HLA identical, but the number of such donors is often limited. It is therefore often necessary to use donors mismatched for at least one HLA A or B antigen. Mismatching for donor antigens that are serologically cross-reactive with recipient antigens can be a successful, albeit imperfect, strategy for selection of partially matched donors.

Interestingly, approximately 25% of platelet transfusions that are mismatched for non-cross-reactive HLA antigens produce satisfactory increments in alloimmunized patients. Older HLA typing evaluations as well as more recent in vitro studies by Liebert and Aster demonstrated that there can be wide variation in the expression of the HLA B12 antigen (more recently separated into its serologic “splits” B44 and 45) on the platelets and lymphocytes from the same individual. Using a quantitative antibody inhibition assay, there was a 35-fold difference in the amount of HLA B12 detectable on the platelets from 25 individuals, although there was little interindividual variation of antigen expression on the lymphocytes from these donors. The absence of this antigen on platelets from individuals whose lymphocytes express HLA B12 could account for many of the successes seen with mismatched platelets.

We therefore conducted a retrospective analysis to determine whether transfusions mismatched for HLA B12 or 44 and 45 can be of benefit to alloimmunized recipients. This could potentially result in considerable expansion of the number of donors available per patient because the HLA B12 group is quite common in that it is detected in approximately 25% of the population.

METHODS

Patient population. The recipient population consisted of patients with documented refractoriness to random donor-platelet transfusion who had lymphocytotoxic antibody detected in their serum using standard cytotoxicity assays. Only transfusions administered to patients who were not seriously infected, with temperature less than 103°F, and without splenomegaly were evaluated. All recipients lacked the HLA B12 group and received single donor-platelet transfusions that were HLA identical except for a single antigen mismatch of HLA B12, 44, or 45. A transfusion from a donor with HLA type (A2, B44; A1, B8) would be an example of such a mismatch. Single donor platelets were collected using the Haemonetics apheresis technology (Haemonetics Corp, Braintree, MA) with or without the surge technique and were administered within six hours of collection.

These data were collected over a 9-year period of time, and 73 different donors provided the platelet transfusions used in this study. In the earlier years of the study, it was not possible to detect HLA B44 or 45, and most donors were typed as HLA B12. More recently the donors were more rigorously classified, with most typed as HLA B44.

Post-transfusion results were standardized for recipient size and the number of platelets transfused using a corrected count increment (CCI), which is the absolute increment × recipient body surface area divided by the number of platelets transfused × 10³. Thus if a transfusion of 4 × 10¹¹ platelets to a 2 m² recipient produced an absolute increment of 40,000/µL, the CCI = 40,000 × 2/4 × 20,000. A successful transfusion was defined as a CCI greater than 7,500 at one hour or, more recently, ten minutes after transfusion.

Lymphocytotoxic cross-matches were done using standard techniques. Platelet cross-matches were performed using a microelisa assay as previously described. A positive cross-match was defined as an absorbance 2 SD greater than the average results of ten normal individuals tested concurrently.
RESULTS

Fifty-four patients received a total of 162 evaluable transfusions. Patients received between one and ten mismatched transfusions with a median number of 2 transfusions per patient.

The overall results are summarized in Table 1. The mean CCI was 12,900 (n = 162, range 0 to 39,500). Overall 111 of the 162 (69%) transfusions produced CCI greater than 7,500. In 57% of the patients, all the transfusions that were administered were successful and produced satisfactory increments. An additional 19% of patients had some transfusions that were successful, while one quarter of patients had no transfusions that were successful. Patients in whom transfusions were successful tended to have received more mismatched transfusions than the other patients. This was because donors were often used repeatedly if their initial transfusion produced satisfactory results. If one considers only the first transfusion of any donor-recipient pair, then the overall success rate was 58% of transfusions.

A number of the recipients had evidence of preformed antibody against the HLA B12 group. The results of transfusion to these patients are shown in Table 2. Satisfactory increments were seen following 23 transfusions to seven patients despite positive in vitro lymphocytotoxic cross-matches between the donor and the recipient. The transfusion course of one such patient is outlined in Fig 1. Similarly, three patients had antibody with specificity against the B12 group that could be detected in vitro. Nonetheless, 11 transfusions to these three patients produced satisfactory increments. In contrast, a total of 14 transfusions to ten other patients with either positive lymphocytotoxic cross-matches or anti-HLA B12 antibodies failed to produce satisfactory increments. These results are compatible with the findings of variable HLA antigen expression on platelets and lymphocytes from the same individual and indicate that this phenomenon may vary widely among donors.

To further assess this possibility, the HLA types and transfusion results from particular donors were analyzed. Of note is that 60 of the 73 donors (82%) were also positive for the HLA A2 antigen. This is a consequence of the well-established linkage disequilibrium between HLA A2 and B12 and suggests that most of these donors had an HLA A2 and B12 haplotype. A similar fraction of the recipients also shared these HLA types. Fifty-four of the 73 different donors donated on only one occasion (38 successful, 16 nonsatisfactory transfusions), and therefore one could not assess whether these donors might be "universal" donors in whom the antigen is poorly expressed on platelets. Variable results were seen in the 19 individuals who donated to more than one patient. With 13 of these donors, all transfusions were successful; mixed results, that is some successful and some nonsuccessful transfusions, were seen with eight donors. Some of these latter individuals donated on only two or three occasions, however, and it is possible that clinical factors may have in part been responsible for these discrepant results.

Because there has been a suggestion that certain HLA types (in particular HLA A2, A3, and A28) may be associated with the lack of expression of B12 on platelets, the HLA types of these 19 donors were analyzed. There appeared to be no particular pattern of the other HLA antigens and whether or not the transfusions from these donors were successful. Thus, although 10/11 donors whose platelets consistently produced good increments were HLA A2 positive, similar fraction of donors whose platelets produced variable results (7/8) were A2 positive. In addition, 13/16 donors whose platelets were ineffective were HLA A2 positive. There were an insufficient number of observations to evaluate the effect of HLA A3 or A28. Lastly, although the presence of HLA A11 has been felt to enhance the expression of HLA B12 on platelets, the single transfusion provided by an HLA A11-positive donor produced excellent results.

Platelet cross-matching was not done as a part of the donor selection process for these patients. A total of 12 cross-matches were done retrospectively using sera from four patients. Satisfactory increments were obtained following six transfusions to two patients in which the cross-matches with the donors' platelets were negative while poor increments were noted following two transfusions with positive cross-matches. Conversely, four false positives were noted with satisfactory increments despite incompatible in vitro cross-matches.

DISCUSSION

Results from a number of laboratories have suggested that there can be a wide range of expression of HLA B12 (B44, B45) antigen on the lymphocytes and platelets from the same individual. Although the quantity of HLA B12 seems to be quite constant on the lymphocytes of multiple individuals, there can be up to 35- to 40-fold variation in the expression of HLA B12 on their platelets. Variable expression of a more modest extent has also been described for HLA B8 and the Bw4 and Bw6 antigen group. In at least one family, Aster et al have demonstrated that this variable expression is an inherited characteristic. Although the mechanism is unknown, there appears to be a rough correlation between the amount of soluble serum B12 antigen and platelet B12 antigen. It has been suggested that platelet HLA antigens are not intrinsic to the platelet but rather represent soluble HLA antigens adsorbed onto the platelet membrane. The direct relationship between serum and platelet HLA are consistent with either of two hypotheses: (1) soluble HLA B12 is reduced in patients with low amounts of platelet B12 because platelets supply the major source of soluble antigen; and (2) the platelets do not have B12 on the surface because there is insufficient soluble antigen to be adsorbed. Experiments in which HLA B12-"negative" platelets are incubated with B12-containing plasma could help address this issue.

<table>
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<tr>
<th>Table 1. Transfusion Results</th>
<th>Median No. Transfusions/Patient</th>
<th>% of Patients</th>
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<tr>
<td>All transfusions successful</td>
<td>57% (31/54)</td>
<td>2 (range 1-10)</td>
</tr>
<tr>
<td>Some transfusions successful</td>
<td>19% (10/54)</td>
<td>5 (range 3-8)</td>
</tr>
<tr>
<td>No transfusions successful</td>
<td>24% (13/54)</td>
<td>1 (range 1-3)</td>
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<tr>
<td>Overall: 111/162 (69%) of transfusions → CCI &gt; 7,500</td>
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CCI = 1/162 (69%) of transfusions

Overall: 1 1

1 of 162 (range 0 to 39,500).

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We have attempted to capitalize on this discrepancy in antigen expression in an effort to improve donor selection for alloimmunized patients. The present data indicate that this can be a very successful approach in that 69% of 162 single donor transfusions mismatched only for the HLA B12 antigen group produced satisfactory post-transfusion platelet count increments in alloimmunized patients. There is very limited previous experience using this approach. In 1972, Thorsby et al. described a single alloimmunized patient who was supported for more than 1 ½ years with platelet transfusions from a donor mismatched only for HLA B12. Satisfactory increments were achieved despite the presence of a positive lymphocytotoxic cross-match between the donor and the patient. Yankee has also described a similar patient. As summarized in Table 2, we also noted satisfactory increments despite the presence of recipient antibody against these antigens in a total of ten patients. The ability of platelets from these donors to circulate normally despite the presence of antibody directed against donor lymphocytes is further support for the in vitro observations of differences in antigen expression on different cells from the same individual. Previous studies in which lymphocytotoxicity cross-matching was used to identify potentially compatible donors have always noted some donor recipient pairs in whom good increments were achieved despite a positive in vitro cross-match. Mismatches for HLA B12 might have accounted for some of these observations.

Although the 69% "success rate" appears superior to other reports in the literature using partially mismatched platelets, there are very few published data with which to directly compare our results. Dahlke et al. have published the largest series in which the results of transfusions mismatched for single antigens have been summarized. However, these authors considered only transfusions that were mismatched for single antigens and that were serologically cross-reactive with recipient antigens. The HLA B12 group is cross-reactive with HLA B-21. Only one of our recipients was B-21-positive, and therefore the present results should be compared with transfusions mismatched for single non-cross-reactive antigens. Using criteria and definitions identical to those used in the current study, Kickler et al. reported that 30 of 44 (68%) of transfusions mismatched for one or two HLA antigens produced satisfactory one-hour post-transfusion increments. These mismatched antigens were selected by avoiding HLA types against which the recipient had developed antibody. In addition, although not explicitly stated, it is likely that some of these were cross-reactive antigens as well. Tosato et al., in a series of 12 highly alloimmunized patients with aplastic anemia, noted that 37% of 20 transfusions mismatched for non-cross-reactive HLA antigens produced "good to excellent" increments. Duquesnoy et al. described 29 alloimmunized patients receiving platelets mismatched for one or two non-cross-reactive antigens. Approximately 60% of these transfusions produced satisfactory increments, although it should be noted that donor-recipient pairs could be reported as often as twice in these data, possibly over-representing the effect of individual compatible donors. In addition, it is likely that the donor selection for certain patients was influenced by prior successful or nonsuccessful experience with other donors of identical HLA type, adding another potential bias in comparing these data. Data from our own institution are not available for comparison because in recent years we have tended to use either donors mismatched for cross-reactive antigens or donors with mismatched antigens selected with consideration of the antibody specificity found in the recipients’ serum.

It would thus appear that the empiric strategy of mismatching for the HLA B12 antigen group produces results that are at least equivalent and almost certainly superior to "random selection" of single antigen mismatches. It was hoped that analysis of other donor HLA antigens would allow identification of patterns of HLA antigens associated with success or failure of platelets mismatched for HLA B12. Despite the large number of transfusions administered in the

<table>
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<th>Positive lymphocytotoxic cross-match</th>
<th>Successful</th>
<th>Not Successful</th>
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<tr>
<td>Anti-HLA B12 (44, 45)</td>
<td>23 transfusions/7 patients</td>
<td>6 transfusions/5 patients</td>
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<tr>
<td></td>
<td>11 transfusions/3 patients</td>
<td>8 transfusions/5 patients</td>
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Fig 1. This alloimmunized patient with acute myeloid leukemia and high levels of lymphocytotoxic antibody (LCT Ab) had satisfactory increments (despite positive lymphocytotoxic cross-matches) following transfusion of platelets from donors A and B, which were mismatched only for HLA B12. Increments deteriorated later in her course as her clinical condition worsened. Donor C was mismatched for two antigens, and these transfusions were not included in the current study.
present study however, only 19 donors donated to more than one patient, and such a pattern could not be identified. Similarly, because more than 80% of the donors were also positive for HLA A2, no relationship could be established between the presence of this antigen and the results of transfusion. Clinical evaluations, which are influenced by the patient’s medical condition and the quality and quantity of recipient antibodies, may also be too crude an assessment of what may be subtle differences in antigen density, however.

The disproportionate incidence of HLA A-2 in both donors and recipients is not unexpected because although the rate of alloimmunization appears to be similar in HLA 2-positive and negative individuals,24 this antigen is found in approximately 50% of the population. Therefore, given the linkage disequilibrium between HLA A2 and B12 (found together in approximately 13% of the population), it would be expected that a high proportion of donors selected for B12 would also be A2 positive. Expressed differently, it would be difficult to find large numbers of donors mismatched for HLA B12 for recipients who lack HLA A2 because there would be relatively few such donors available, even in a large donor pool.

It might be hoped that platelet cross-matching of donors and recipients would be more predictive of success with this single mismatched antigen. Our experience in this regard is limited. False-positive results were noted following four of 12 such cross-matches. False positives (ie, incompatible in vitro cross-match with good post-transfusion results) have been noted in most prospective and retrospective studies of platelet cross-matching as a means of donor selection. Kickler et al26 noted nine of 35 positive cross-matches with good post-transfusion increments in a retrospective analysis of a series of platelet transfusions to alloimmunized patients. Similar findings were noted by the same group following nine of 23 transfusions from donors prospectively identified using a radiolabeled antoglobulin assay.27 McFarland and Aster also noted three of nine false positives using the most sensitive of a series of four different cross-matching approaches.28 These discrepancies are probably a consequence of the variable sensitivity of the large variety of available platelet cross-matching techniques28 but may also indicate that the platelet-bound immunoglobulin detected in vitro may not be sufficient to promote rapid clearance of such platelets when administered in vivo.

In summary, the current data represent a practical application of the biologically fascinating observation of variable antigen expression to the clinical problem of platelet transfusion for alloimmunized patients. Almost 70% of transfusions mismatched for HLA B12 can produce satisfactory increments in alloimmunized patients and support this strategy as a means to select donors for such individuals. Because the HLA B12 group is found in approximately 20% to 25% of the population, mismatching for this antigen potentially substantially expands the number of donors available for patients otherwise refractory to platelet transfusion.29 30

REFERENCES

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